RUTIN

James F. Couch and Charles F. Krewson

Eastern Regional Research Laboratory[1]
Philadelphia, Pennsylvania

Indications that rutin is an effective therapeutic agent in the treatment of increased capillary fragility (1) have stimulated renewed interest in this substance.

Rutin ($C_{27}H_{30}O_{16}.3H_2O$) is chemically a rhamno-glucoside of quercetin and thus a derivative of flavonol (5, 6). It occurs as a bright yellow powder consisting of masses of needle-shaped crystals melting at 192-196° C. and decomposing sharply at 214°. It is difficultly soluble in cold water; approximately 0.13 g. per liter at 20°, and more soluble in boiling water; soluble in methanol, ethanol, pyridine, acetone, ethyl acetate, and alkalies; and insoluble in chloroform, ether, and the hydrocarbons. On hydrolysis with dilute acids rutin yields quercetin, glucose, and rhamnose:

$$C_{27}H_{30}O_{16} + 2H_2O = C_{15}H_{10}O_7 + C_6H_{12}O_6 + C_6H_{12}O_5$$

Rutin darkens on exposure to light and should be preserved in bottles of dark glass or in a dark place.

Rutin is non-toxic. Experiments conducted by the Bureau of Animal Industry in collaboration with the Eastern Regional Research Laboratory have shown the absence of acute or chronic toxicity to laboratory animals. Patients treated daily for periods of several months have exhibited no symptoms that could be attributed to the rutin administered to them.

---

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture.

Occurrence

Rutin occurs in several plants. At this Laboratory we have been interested in this glucoside as a byproduct in the industrial utilization of tobacco. Rutin was first reported in flue-cured tobacco by Hasegawa (2). Neuberg and Kobel (3) isolated rutin from green tobacco and reported that it is oxidized during the process of curing, contributing to the deep-brown color of air-cured tobacco. Later (4) they made enzyme preparations from green tobaccos of different origins and showed that these were capable, under proper conditions, of catalyzing the oxidation of rutin to brown pigments by hydrogen peroxide.

Results obtained in this Laboratory are consistent with the views of Neuberg and Kobel. Many samples of tobaccos have been investigated, but no rutin has been obtained from any air-cured type. Burley, Pennsylvania cigar filler, Green River, Maryland, fire-cured, one-sucker, and Nicotiana rustica were separately examined, and no rutin was found.

Our results indicate that rutin may be obtained only from flue-cured leaf. The quantity obtainable varies with the quality of the leaf and probably with its age. The yields have ranged from 0.008 to 0.61%, averaging about 0.4% for good-quality leaf. The best yields are obtained from leaf of high quality. For manufacture on a scale larger than laboratory lots, we have used U. S. Grade C3L or better. The stems, even from high-grade leaf, have furnished no rutin.

Extraction

The best process for the extraction of rutin from flue-cured tobacco is percolation with alcohol. The air-dried leaf in coarse powder - number 20 is satisfactory - is moistened and packed into a percolator and extracted with alcohol in the usual way. In our experiments we have not used hot percolation on large lots, but there is no obvious reason why this should not be employed if desired.

The alcohol is removed from the extract by distillation under reduced pressure, and the residual mass, consisting of fats, resins, sugars, coloring matters, acids and other constituents in addition to rutin, is extracted with boiling distilled water as long as soluble matter is removed.

The water extracts are mixed and filtered. On standing, the filtrate slowly deposits bright-yellow warty masses on the walls and bottom of the container, which consist of masses of microscopic needle-shaped crystals of rutin. These crystals are often of high purity but sometimes are accompanied by a brownish impurity. The latter is insoluble in methyl and ethyl alcohols and in boiling water.

The crude rutin may be purified by recrystallization from boiling water or from 50-percent ethyl alcohol. Boiling water furnishes more readily a pure product, but the solubility of rutin in this solvent is so small, about 5.5 g. per liter at 100°, that large-scale operation presents difficulties, owing to the large volumes of solution that must be handled.

A convenient method for recrystallization that has been successful in this Laboratory is conducted as follows: Rutin (10 g.) is dissolved in hot alcohol (200 ml.), and the solution is added to boiling water (750 to 1000 ml.) and filtered if necessary. On cooling, the solution deposits flocculent masses of crystalline rutin, which may be filtered by suction, washed with cold water, and dried at 110°. Several recrystallizations are usually required to yield a product that melts above 192°.

A modification of the extraction process described above, which has been developed at the Eastern Regional Research Laboratory, permits the greater part of the rutin to be recovered in a shorter time. The new procedure involves fractional percolation in which the first and most concentrated percolates are worked up as they are obtained and independently of the later and weaker percolates. Working with batches of 44 pounds (20 kg.) of tobacco, which require

the passage of 60 gallons of menstruum for complete extraction, the percolate was collected first in fractions of 10, 10 and 40 gallons, and later in fractions of 20 and 40 gallons. Two batches of 22 pounds each were extracted with methyl alcohol, and the size of the fractions of percolate was halved to correspond with the half-portion of tobacco extracted. The results of this manipulation showed that the first third of the total percolate contained 75 to 100 per cent of the total rutin obtained. The rutin from this first third was purified and ready for use before the percolation of the second fraction was completed. Under continuous operation the second fraction would not be worked up to rutin but would be used to moisten and extract a second batch of tobacco. In this way economy of time, material, and equipment could be effected.

Although rutin is very soluble in methanol, the yields are lower when this solvent is used for extraction as compared with ethanol. Loss of rutin occurs when methanol is used for recrystallizing crude rutin, and there is production of excessive quantities of brownish resinous material, which appears to be due to some reaction between rutin and the solvent. The same phenomenon probably occurs when methanol is used to extract tobacco and results in lower yields by destruction of the rutin.

References

(1) Griffith, J. Q., Jr., Couch, J. F., and Lindauer, M. A., Proc. Soc. Exp. Biol. and Med. 55: 228-9 (1944).

(2) Hasegawa, H., J. Agric. Chem. Soc. Japan, 7: 1036-49 (1931).

(3) Neuberg, C., and Kobel, M., Naturwiss, 23: 800-1 (1935).

(4) Neuberg, C., and Kobel, M., Enzymologia 1; 177-82 (1936).

(5) Perkin, A. G., J. Chem. Soc. 97: 1776-7 (1910).

(6) Sando, C. E., and Lloyd, J. U., J. Biol. Chem. 58: 737-45 (1924).